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DESCRIPTION

METASTASIS INDUCING DNA'S

ins 1

The present invention relates to metastasis inducing DNA's, a method of identifying such DNA's, and their use in diagnosis and therapy.

ins 2

Most cancers are thought to be due to alterations in specific genes caused either by mutation making their gene-product in some way more effective or by over expression of a normal gene giving an enhanced effect. These oncogenes have largely been identified by introducing gene-length fragments of DNA from human cancers into a mouse fibroblast cell line, in culture, and selecting those cell lines that grow in an uncontrolled manner in liquid or semi-solid medium. The oncogenes themselves have been isolated by cloning the human DNA fragments away from the mouse DNA by standard recombinatorial techniques. Alternatively mutations can arise in genes that suppress their own activity such as, for example, p53 or Rb or which suppress the levels of their products such as, for example NM-23. These are referred to as tumour suppressor oncogenes. In the commonly-occurring cancers, it is believed that between 5 and 7 such changes in oncogenes or tumour suppressor oncogenes are required to produce a full-blown cancer.

WO 86/03226 discloses a method for detecting a discrete, transmissible mammalian gene associated with tumour metastasis. The method uses a non-syngeneic

system. The teaching was later retracted - Proc Nat. Acad. Sci USA, 1988, 85 5581.

WO 94/28129 identifies a tumour metastasis gene of 2858 base pairs which codes for a protein which is expressed in malignant human tumours and their metastasis. The method used to identify it used a non-syngeneic system employing nude (defective) mice.

Cancer research 54, 2785-2793 (1994) is a paper by the applicants. It discloses a method for showing the presence of metastasis inducing DNA. No disclosure is, however, made of how to recover the sequences for identification.

Cancer research 54 832-837 (1994) is a paper suggesting that antisense OPN DNA expression was associated with reduced tumorigenicity of these cells in the flanks and in lungs. The paper does not measure or investigate metastasis as such.

EP 0607054 disclosures a process for constructing a cDNA library. It described a method, using linkers and PCR for identifying signal peptides. The application is not to metastasis at all and the approach uses expression vectors for detection.

The major forms of cancer, including breast cancer, lung cancer and colonic cancer cannot be cured effectively because, although the current therapies may

be effective against the primary tumours, they are largely ineffective against the disseminating or metastasizing cells, which ultimately kill the patient. Despite the enormous effort in cancer research very little is known at the molecular level about the most important life-threatening process, that of metastasis. Most of the oncogenes and suppressor oncogenes that have been discovered have been found from their ability to promote uncontrolled growth of the mouse fibroblast cell line. The major problem in this field is that determining cell growth does not give a measure of the process of metastasis. In fact, although uncontrolled growth is an important aspect of the initial events in the development of a cancer, the rate of growth of distant metastases can be remarkably slow. Hence the process of metastasis is largely independent of processes involving cell-growth, except in its final phases. Therefore, it is unlikely that oncogenes and tumour suppressor oncogenes will have much involvement in the process of metastasis and be useful diagnostic or therapeutic targets for control and elimination of metastatic disease.

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It is one object of the present invention to identify DNA comprising, consisting of or containing sequences involved in metastasis, hereinafter referred to as metastasis inducing DNA's or Met-DNA's for short.

According to a first aspect of the present

invention there is provided a method of screening and recovering a regulatory DNA capable of inducing metastasis comprising the steps of:

i. transferring tagged fragments of a human DNA from malignant, metastatic cancer cells into a cell line that produces only benign, non-metastasizing tumours when injected into a syngeneic animal;

ii. injecting the transformed cells into the syngeneic animal;

iii. selecting those animals in which metastasizing tumours have been identified; and

iv. recovering the regulatory DNA capable of inducing metastasis therefrom.

Preferably the DNA fragments transferred in step 1 are fragments of from 0.1 to 50 kilo base-pairs, more preferably 0.5 to 50 kilo base-pairs.

Preferably the cell line that produces only benign non-metastasizing tumours when injected into a syngeneic animal is a rat mammary epithelial cell line, such as, for example Rama 37.

Preferably the fragments of human DNA from malignant, metastatic cancer cells are tagged to assist in their removal or insertion from or into a host or vector, such as, for example, the oligonucleotide tag illustrated in Fig. 1. This tagging procedure overcomes the problem of identifying the inserted human DNA sequences in the rat genome of the transfected rat cells. Human-specific repetitive DNA (Alu) sequences are spaced sufficiently in the human genome that in many human DNA

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fragments of this size they will be absent.

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In one embodiment, fragments of human DNA from malignant, metastatic breast cancer cells are introduced into a rat mammary epithelial cell line Rama 37 which produces only benign, nonmetastasizing tumours when injected into syngeneic rats.

By way of example only, the transfer of restriction-enzyme *Hind*III-fragmented DNA from malignant metastatic rat and human breast cancer cell lines into a benign Rama 37 cell line produced a small proportion (1-3%) of transformants which, when reintroduced into the syngeneic rats, caused these cells to metastasise, principally to the local lymph nodes and lungs. In contrast, fragmented DNA from nonmetastatic cells and the standard oncogenes (Ha-ras, Middle T Antigen gene, and Large T Antigen gene) produced no metastasizing transformants. The latter result confirms the non involvement of such oncogenes in the metastatic process *per se*. However, the fact that metastasis can be transferred in a genetically dominant manner suggests that other dominantly-acting DNA fragments are largely responsible for this process. The full results of the above experiments are shown in table 1, which shows the incidence of tumours and metastases for Rama 37 transfected cell lines.

The column headed "cells injected" gives the cell type in short hand, and full details are given

below:

Rama 37 are Rat mammary 37 benign cells; R37-Ca2-LT1 is a cell line from a lung metastasis of Rama 37 cells transfected with fragmented DNA from the metastatic breast carcinoma cell line Ca2-83 (Cancer Res 54 2785-2795, 1994); B-T1 is a cell line from a primary tumour of Rama 37 cells transfected with fragmented DNA from the benign breast cell line HMT-3522 (Cancer Res. 54 2785-2795, 1994); R37-Ca2-HT is a cell line of Rama 37 cells transfected with tagged DNA fragments from metastatic transformant R37-Ca2-LT1; R37-Ca2-H is a cell line of Rama 37 cells transfected with untagged DNA fragments from metastatic transformant R37-Ca2-LT1; R37-B-HT is a cell line of Rama 37 cells transfected with tagged DNA fragments from the benign transformant B-T1 as a control; R37-F1 is a cell line of Rama 37 transfected with PCR fragment F1 from a cell line of a lung metastasis of R37-Ca2-HT; and R37-F2 is a cell line of Rama 37 transfected with PCR fragment F2 from the same cell line of a lung metastasis of R37-Ca2-HT.

The b annotation in the column headed metastases identifies the transfecting DNA's giving rise to significantly more metastasis than Rama 37 cells (P<0.05, Fisher exact test). The animals were autopsied after 3 months.

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To aid the rescue of metastasis-inducing human DNA sequences from the rat transformant cell lines, all

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the HindIII-fragmented DNA's from one such metastatic transformant, R37-Ca2-LT1 (Table 1) were tagged at both ends with double-stranded synthetic oligonucleotides that provide restriction enzyme and unique PCR primer sites. These are shown in Fig. 1 The tagged DNA fragments include 4 restriction sites: *Sfi*I and *Not*I, a defective *Hind*III site at the 3' end for linking to the *Hind*III sites at the ends of the human DNA fragments, thereby destroying it, and an internal *Hind*III site located near to the 5' end, which when cut after ligation generated new fragments with *Hind*III ends. The fragments were transfected into the parental Rama 37 cells, and after transfer of the cells to the mammary glands of syngeneic rats, metastatic cell lines were isolated from the resultant rat lung metastases. The tagged, fragmented DNA incorporated into the metastatic transfected Rama 37 cell lines was directly amplified between the tags by PCR and yielded bands at about 1300 to 1500 bp that were responsible for the metastasizing ability of the transfected cells. These results are shown in Fig. 2 which shows the DNA fragments produced by PCR of metastatic transformants. Two new cell lines, established from the culture of lung metastases of R37-Ca2-HT (tagged, metastatic DNA transformant) and R37-Ca2-H (untagged, metastatic DNA transformant) (see Table 1) in rats were termed HTLu and HLu, respectively. They were run against the tagged benign transformant cell

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line R37-B-HT and the tagged metastatic transformant R37-Ca2HT. Cellular DNA was amplified by PCR using a short oligonucleotide primer of 22 bp from positions 3-24 of the tag sequence as shown in Fig. 1. Compared with the control DNA's from HLu and B-HT cells, two extra bands, F1 and F2, of about 1300 bp and 1500 bp respectively, were specifically amplified from genomic DNA of the Ca2-HT and HTLu cells when PCRed DNA samples were run on 0.8% agarose gels containing ethidium bromide and photographed in U.V. light. The fluorescent bands of DNA are shown in negative imaging for clarity. Cloning of these pooled DNA's yielded six independent fragments and the results are illustrated in Fig. 3. Fig. 3 shows pBluescript clones of metastatic DNA fragments F1 plus F2. The two broad PCR DNA fragments F1 and F2 were excised from the gel in Fig. 2, combined, and cloned directly using the AT procedure into a suitably modified pBluescript vector and the clones of recombinant vectors were cut with *Hind*III to excise the cloned fragments. These cut recombinant vectors were analysed on a 0.8% agarose gel containing ethidium bromide and photographed in U.V. light. The sequences of some clones eg. C10 and C9-DNA's were identical; the six independent sequences arose from clones numbered C2,C5,C6,C9,C12 and C20 and hence are referred to as C2-DNA, C5-DNA etc as shown in Fig. 3. The position of the vector (Vec) DNA and insert (Ins) DNA are indicated and a standard molecular weight

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ladder in kilobase pairs (kbp) is shown in lane M. Transfection of these cloned DNA fragments singly into the parental benign cell line confirmed that all fragments (C2,C5,C6,C9,C12 and C20-DNA's) produce metastases. These are shown in Table 2 which tabulates the incidence of tumours and metastases for Rama 37 cells transfected with cloned Met-DNA's. The superscript a - e indicate:

[a]Benign nonmetastatic Rama 37 cells were transfected with pSV*neo* or with pSV2*neo* and different independently-cloned inserts of the pBluescript library of pooled F1- and F2-DNAs termed C2-DNA etc. or with a cylomegalovirus expression vector pBKCMV (CMV-1) or with the CDNA for osteopontin (*opn*) cloned into the same expression vector pBKCMV*opn* (OPN-1).

[b]Transfectants were tested for their level of *opn* mRNA relative to that in Rama 37 cells by Northern hybridisations to *opn* CDNA using a Shimadzu CS9000 scanning densitomer. RNA loading levels were standardised with respect to a 36B4 ribosomal protein constitutive probe.

[c]Transfectants were tested in the mammary glands of rats for the percentage (%) of tumour-bearing animals with metastases in the lungs after 3 months. The incidence of tumours produced by all transfectants was 100%.

[d]Significantly higher levels than for Rama 37

cells (P<0.05;Mann Whitney U test).

[e]Significantly more metastases than for Rama 37 cells (P<0.05;Fisher exact test).

Thus Koch's postulate has been satisfied for all metastasis-inducing-DNA's (Met-DNA's) in this system.

Southern hybridisations and PCR amplifications have established that the Met-DNA's are specifically present in their respective transformants.

Fig. 4 shows detection of C9-DNA in transformant cell lines. Cellular DNA was isolated from (A) a cell line from a lung metastasis produced by injection of C9-DNA transfected Rama 37 cells in rats; (B) C9-DNA transfected Rama 37 cells (see Fig. 3 and Table 2); (C) benign Rama 37 cells; (D) benign BT-1 cells (see Table 1). These DNA's were digested with *Hind*III and the digested DNA was analysed on 0.8% agarose gels either by (A) Southern blotting to a probe of $[^{32}P]$ radioactively labelled C9-DNA, and the radioactivity visualised on X-ray film or (B) by PCR using the 17 oligonucleotide fragment from either end of the C9-DNA as primers and run with a standard molecular weight marker ladder. The newly synthesised DNA in B is visualised by fluorescence of the ethidium bromide in the gel in U.V. light.

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Surprisingly, the sequences of these Met-DNA's (sequence 1 to 6 hereafter), although human in origin, do not correspond to known genes and most do not include any

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known open reading frames. Furthermore none of these Met-DNA's are expressed as mRNAs in their transformants and hence are not dominantly-acting oncogenes. They therefore contain entirely novel short stretches of regulatory DNA capable of inducing metastasis.

The state of the Met-DNA's has been investigated in the metastasizing transformant cells. Bands of greater than 23kbp which hybridise to the C9-DNA probe have been obtained from *Hind*III digested C9-DNA transformants, and pulsed-field gel electrophoresis yields multiple bands of about 16-48kbp after similar digestions as shown in Figure 5a-d.

Fig. 5 shows the detection of Met-DNA in transformant cells. The cellular DNA was isolated from :(A) a cell line from a lung metastasis produced by injection into rats of C9-DNA transfected Rama 37 cells; (B) C9-DNA transfected Rama 37 cells; (C) benign Rama 37 cells; (D) benign primary tumours of R37-BT-1 cells. These DNAs were digested with excess *Hind*III and the digested DNA was analysed on agarose gel (a) with continuous electric field; (b) with a pulsed electric field; or (c) by PCR using 17 mer oligonucleotide primers from each end of the C9-DNA; (d) These DNAs were also digested with excess *Eco*R1 and analysed on agarose gels with a continuous electric field. The resultant gels were either (a.b.d) Southern blotted to a probe of [^{32}P] C9-DNA without tags and the radioactivity visualised on

X-ray film or (c) the newly synthesized DNA was visualised by fluorescence of the bound ethidium bromide in U.V. light. Controls with (a) C9 DNA in lane P and (c) standard molecular weight marker ladder in kilobase paris (kbp) in lane M were also run. This result strongly suggests that the flanking *Hind*III sites have been destroyed by the transfection/integration process. The highest 48kbp band is preferentially retained by the cell line isolated from a lung metastasis (Figure 5b); thus is is likely that this represents most of the metastasis-inducing DNA (Table 2). The C9-DNA transfectants contain about 100 copies per haploid genome of C9-DNA when compared with a single copy (Figure 5a, lane P) 10 copy and a 100 copy DNA control. PCR amplification of the integrated DNA using primers complementary to the cDNA adjacent to the untagged ends of C9-DNA produces a single 1kbp product showing that the integrity between the primer sites has been maintained (Figure 5c). However, digestion of the DNA of C9-DNA transfectants with *Eco*R1 (which cuts once internally within the C9-DNA) and hybridisation with a C9-DNA specific probe yields predominantly a 1kbp band of similar size to the original C9-DNA insert (Figure 5d). This 1kbp band probably arises from the digestion of tandem repeats of C9-DNA. Similar results have been obtained with C2, C5, C6, C12 and C20-DNAs.

The occurrence of C9-DNA has been investigated

in pilot studies in the DNA of human breast cancers. Hybridisation of C9-DNA occurs to *Hind*III-digested DNA from 4 out of the 9 breast tumours tested, whereas no hybridisation signal is detected from similarly-digested DNA from normal human breast or colon tissue. In this case a single hybridising band of 1000bp is detected (Figure 6).

Figure 6 illustrates detection of C9-DNA in human breast tumours. Cellular DNA was isolated from a selection of nine randomly-picked human breast tumours numbered 14-130 and from normal breast and colon tissue together with C9-DNA as a control. These DNAs were digested with an excess of *Hind*III and the digested DNA was analysed on agarose gels, Southern blotted on to a filter and hybridised to a probe of [^{32}P]C9-DNA without tags and the radioactivity visualised on X-ray film. Similar results have been obtained using PCR for C9-DNA.

According to a second aspect of the present invention there is provided a regulatory DNA capable of inducing metastasis consisting essentially of a human DNA fragment of less than 1.6 kilobase pair in length obtained from a malignant, metastasis cancer cell.

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According to a third aspect of the present invention there are provided DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 1:

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C2

CTTCCTTGGT GCTCTATGTC TTGCCTCTCC CCTTCTCCAG TCCCATTAAG CCATAACCAT
CTTGACAGAC TCTGGGACAG TCCCCTCTGC TCTCCTGTTG GCGCCTGAGT CCCTTTTTGC
CTGAGGACCC TTCACGTAGC CTCCCATCTG GATGACCTAG TAGAAGACGT GGGAAGTTGT
CACACTCAGG TAACTGAGCA GAGCTCAGAG ATTTAAAGTG AGTCTGGGGA GCCTCGAGGA
TTGATCTGCT GCCTTAAAAA GCCAATTGGA TGACTAACCC AGACTATTGT CACTTTAGGT
GGGAAGTCAC TAGCATATCT GATGGGTCAC ATCTGAGAAA GGTTTCTAGC AGTGGTGGCC
TTGTGTGAGC AGCATGGCGT GTATCATGGT GTGCAGCATA CTCAGGCTGC TTGCAACACT
CGAGGCTCTT CTTCAGTATT AGGGGAACCA CTGGTGTTSG AACATGGTCC AAGAATACAG
TCATGTGAGG AGAATCCCAA TGCGTCAGGA GAAAACGAGA GTCTGTGACC TCCATTCTTC
AAGATACAGA ATTATTCTTG GACTGTGTTT TCATGCTCCT TGTGGATGGG AGTGAGTTTA
CTTCAGGTTA ATCAGCATTG CTTACTGTTG GTATTCAAGT AAATGCTTAA ATTATCCTGG
ATATACCTCT GTGGGAAGCA GGTTTTTGAT ACATGCAGCT TGTCCTTGTG ATTGATACTG
CTTGAACTCA AGAGAACTTT GCTCATGTGA TCTTTCTTAA CCGATGGAGT AGAAACTGTC
TGATGCTCTC AATAAAGTTG GCTCTTGCAC GAGACGTTAG TCTGTCCTGT TTATCTGCTC
CATTCTTCCG CTCCCACGGC CTCTACAGCA CTAAACCCAC CACCGATAGA CTCAGTCTTT
CACTGACAAA CATCACCAGA GGCTCTTAAC TGAGATTATA AACTGTTACT AGATGATGGG
TGGAATCGCT CCCCAGAAAC ATAAACATTT ACTTGGAGAA CTCAAGACCC CTTTGTAGAC
ATAACTCCCA TGGT

According to a fourth aspect of the present invention there are provided DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 2:

C5

ATTGCTGTGA GCCTATTAGC GACATTTGGT GACGCCCCTT TTAAGGGGGT AGATACAAAG
AATGGGTTGA AATTCTGTGC CACAAACGCT CTCCATGTTT TCACAATTAC ACTTGCAACC
TGTGGTCAGC AGCCAGAATT TAGGGATGTG ATGGGACAGG GTCGGGGAAA GAAGGAGAAG
GGTAAAGGAA AGACAGCACG TTAAAGTCCA AACAGCTCCA GGAGACTATC TGTAGAAATA
ACATCAGACC ATGAGGAGAA TTGATATCAT TGTTTTTCAA TGGGTATCGC CAAGGGAACT
TTCCATCTGA TTAAAAATAA TTACTGCTGG CACTAAATCC AATTGGAAAT GCCCCACACA
ATTTATCTTC CACTTCATGC TGCTACCATA TGCCTGACGT GGCGGAGCAG AAGCATTCCC
TCCCGTTCTG ATAAATAGTA CTTTGTAAAT ATTTGGAGAC GGGAGCTCTG GTGACAGGGA
ACACGTACAA ACCGGCCTGT TTATCATGTT CCCGATAGAG GCCCTCTTTG ACGTACAGGA
CCCCAAAACA GTCAGGATGC TGTGAATTTC CTTCCATGAA GCCTTGTTCA CAATTAGCAA
CCATTGGAGG AAGCAGGCTG CACTGTCTAC CACAAGTGGC ACTTTCCAAA GAGCACACAT
ATATTGGAGC AAGACATTTT GCTGGCTGAC TGGTGCTGTG TAAGCTGATA AACTGCTATA
TTTATTAAAC TGGCTTTTCT TTGAACACCC CACTCAAGGA AAAAAAAACA CACTTAGGGT
GACATTATTT GGAGATGAAG TCTTTATAGA GATGCTTAAG TTTAAACGAG ACTTTTAAAG
CCGGCTCTAT TCCATTTAAT GAATGGTGTC CCTACAAAGG AAGAAACTGG GACAGAGGTA
TGTACACTTG TGTGTGTGTG AGAGACAACG TGAGGAGCTG AAGAGGAGCA CGTACAAGTC
AGAGAAAGGC TGACCCTTAT TCACACTGAG CAAACCAGTC ATGTGTGGGT CGATAGATGA
GAGTATCCCC CAAGACTCAC ACATTCGAAC GCTTGGTC.

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According to a fifth aspect of the present invention there are provided DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 3:

C6

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AGGACCAGAG TTCACATCCC ATCAAATGGC CCAGAAGGTT TTAATGCTGT CTTTTGGCCC
AGGGGCGAAC TGCACACACA TGTGCACATA CACTTACAGA GACACACATT CAGCAGCATA
AGAACACAAT CACAAATAAA AAAAATCTTG AAAAATTTTA AGCTAAAATT GTTAAGAAAT
AACATATATA CAATTTTTCT TTATTTTTTT AAAGATTTAT TTATTTAATG TATATGAGTA
CACTGCCTCT CCCTCCAGAC ATAGCAGTAC AGGGCATCGG ATCCCATTAC AGATGGTTGT
GAGCCACCAT GTGGTTTCAC AGATGGTTGT GAGCCACCAT GTGGTTTCAG GAATTGAACT
CAGGACCTTT GGAAGAGCAG TCAGTGCTCT TAACCTCTAA GCCATCTCTC CTGACCCTTA
TATACAATTT TAATGCTACG TACACACAAC TTCTCTTTCC TTTAATGGTT GAGATTTTTG
TCTGGAGAAG TAAGAATAAA GGAGGGAAAG AACATTGCTT TCACATTGCA CCAGTGGGAA
CAGCGTGTTT AAAGTAGGAA TGCCATGAAA TGACTGGCCT GCCTTCTCAT TACTGTTCCT
CCCACTCCTC CTTTTAACTG GAGCTCCTTT ATCTAATTTA TTAGTTTGAC GATACCCAGG
GTTTTCTTCT GTTTTGATCT TTTTAAGACA GAGACTCACC ATATAGCCCT GGCTGGCCTG
AAGCTCACTA TGTAGACCAG TCTGGCCTTG AACTCAAAGG AGATCTATCT GCTTCCTAGT
GCTGGGATTA AAGGCTTGTG CTACCAAGTC TGGTCTGAGG CTTTGGAGCA GCCTCGGTTT
TGGCCTTCTT TAAGGATCTC TAAGCTAGCA GTAAGTAGCC TAGCCATGCT GTTGTAGGAA
GTTGTTCGTT CATCCTGGCT CCAGCACAAA GGCAGTCACT AAACGTCGGC CTCATTTCAT
CAGAGCTGAA TGCAAATTCC TTGTGCTCTT CCTGTGTCCT CCTGGAAC
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According to a sixth aspect of the present invention there are provided DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 4:

C9

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AGTTGGGGAC ACAGCTTGCT TGATTAAGAT GTTTCTTGGG AAAAGGAGTT AAGCCTAATG
ATTTCCAATG GAAAGGACTG CTAATTGGGG AGGCAATGTT GCTTAATTGG GACACCTGCG
GGTAATTAAA AGCTCTCTCC CAGTGGCCTT TCCTGTTTTT GGCTCTGGGA GGCGAAGGCA
TTGAGAGGGA TGCAGGCATT CTAAGGGCTG GTTCTTGGTT TCTCCCTTCC CCTCTGTCCA
AACTCAGTGA GGTATCCCTG TCTGTGCTGT CCTTAGAGTG CCGTCCTGAG GCCTTGGTGA
GTTAAGGTCT CTGGATCTGA GCTGCCTCAG GGAAACGCAT GAGCTCATTG GAAAGGGGAG
AACCAGGCAA AGGTGTTGGC TGTGACCTCA GAATTCTGAG GGGCAAAGGT TCAAGGCTAA
CTCTCATTAT AGAGCAAGTT TGAGACTGGC CTGGGAACAA AAATATAAAG TGAGTGAGGT
CATATGACAG CACCTGAGGA GTCCTGTCCC TAGAGATCAT AAGGACCTGG CTGCTGGGGA
CTTGTTGCAG ATGGCACTTT GTGTCGAGAG AGGGGACCTG CCCCAGCATG GGAGGCCCTG
GAAGATCCTC TGGATTAACT GTGAACACTG ATTGCTGCTT TATACCTGGA GTTGTGCTGT
TATCTGGTAC ACATCTGCTG GGTGAATGAG TTCATGGGCT TTATTTCAGT GAGGTATTTA
CCTGAGGAGA AAGAAGGACT GGTGCCACAA AGCACAGCTT TTAAATCTGT GGGTTGTGAC
CCATTATGGA CTATCATAAC TGAGTGCAGG TATCAAGAAT ACTTTAGCAG GTGGTAAAAA
GATTTTTGAA TGCGCAACGA CCAAAACTGA ACTCAAAAAT CAAGCATGGC ATGGATCCTG
GGTGCTCCTG GAAGCACTTG CCTTTACTGC ATTGTGCGAC TTGACGGTAG CCTTGGTTCT
GAATGCACAA CACGTGGGCT TTGGGCTGCA CAGGCCACCA CGCCGTGCCT GAAACACCTC
AGCTCAGGTT TGTGGCTATG TCCTATGACT TGGACTTACT TTTATTGCAC ATATAAATAT
TTTCCTGC
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According to a seventh aspect of the present invention there are provided DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 5:

C12

GAGGGGGTGG TGGCACAGTT ATGTTTTTGT AGGAAGGGTT CCATGAACCT CAGCAGAGCT
CGGGTTAGAA ATTTAAAAGC CCTGAGGGGA ATTTTTTTTT TAAATCGCTA TGAATCTGAC
ATGAGAAAAA CAGATCAGAA ACGTTCTTGT GCTTCAGAAA AGGACAAGTG TGTGAGCTAA
CAGACTGCAC ACTGGTGTTC GAGGCACATC TGGATCACAG GAGCGTCAGA TAATGTCCCC
AAAGGTAAAT GCATTTGCTT GCACAGTACC GAGTGTGGTG GGGGGTGCCT ACAGCCCAGC
GGTTCTCAAC CTTCCTGATG CTTCGACCCT TTAATACAGT GCCTCATGCT CTGGTGACCT
CCCCAACCTT AAAATTATTT TTGTTGCTGT TCATAACTGT GATTTTGATA CTGTTATGAA
TTGTAATATA AATAATTTTG AAGAAAGAGG TTTGCCAAGG GTTTGAGAAC TGCTGTTCTA
GCCCCACGTG GATGGTTTTT CGTCATTTGG GGTTTTTATG AGGCAGAGTC TTATGTAGCC
CAGGCTAGCA GCCTAGAATG TGCTACTTAG CTGAGGAATA ACCTTGGAAC TTCTGAGGAC
TGGAGAGACT GGCTTAGTCC TCAAGAAACT GGAAATAGCT GGAGTTTGGC TACTTGTGGG
TTCCTTTTTC TTCAAACCTT TTCTACTCTT TTTCCACCCT GTCGGCCCCC TAACACTAAA
TAAGAAAGAG AAAGGGGAGC ATAGAGGGGA AAAGAAACCC CTGAATAACG TCAGTAGTTG
GCAAAGGGGG GTGACATATG TTGTCATTAG ACCACATCCT GGTGATTAAG GGGAGTCAAG
TTCCTTGGGG CAAGTTTGAT CTTTCGTGTA ACGATATCTA ATTTCTTCTC CCTGTTGCTT
CGTCTTTGTG AACAACGACT TGATAACCCA CAATGGACCA TCAACCAACC AACCAACCAT

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According to a eighth aspect of the present invention there are provided DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 6:

C20

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TTGTCTCTGG TGTTACTTGT TTTCCCATTT CTGACAGTGG TTTGACCTT CTATACGCCT
GTGTGTCAGG AGTGCTGTAG ACCTATTTTC CTGTTTTCTT TCAGCCAGTT ACAGGAACAG
AGTGTTCTAC TGTCAGATGT GTAGCTGTTC CTGTCCACTG ACTTTCAAGC TGTCTCTGTG
TGCAGGAACC AGAAGGGCCT GTCCCTACTT CTACTGGGCC CCTACGCACA GGGGGCCTAG
ATGGTGCTAG GTGTTTTCCT CTAGAGCCTG AAATGTGGGC AGAGAGTAGT CTCCTCTGGT
TTCCTAGGTA TGTCTTCCCC TCTGAAGGTC TAGCTCTCCC TTCCATGGGA TATGGGTGCA
GGGAGCTGTT TGACCAGGTC CTCTCAAATC CGGGTGCAGT CTGGACCGCA GGCTCCTGTA
GCTTGCCTGC TGCAATCTTC CCGCACCCAG AGGCACCCAA GTTTCCTCTT GGGCCAAGGA
TGTGGGCAAA GGTGGGCAGA AGTGGCAATC TCTCCTGCCC TAGCGTCTCA GGATTGCCCT
CACTTCTGGG CAATCCGCTC TCTCTTCCAC AGGGTTTGGG AGCAGGGAGC TGTGGGCCGG
TATCAGGCAA AGGTTTGAGG CAACCAGTTA GAAACTGGAA GTGTCAGGTC CCAGAGGAAT
TTTGCCTTTG TGTGTCCTGA GTCCACCAGG CAGGTCACTT GGAGCAGAAA AATTGGTTTT
CCCCTCGGTC TCAGGCCTGA AGTTGCACCT CAGGGTTGGC TTTCAGCTGT ACCTGTGGAA
AGTATGGTTT TAAAAATCTA AGATAGCTAT CATGCAGCAA GGCTTGTGTA AAATGTCTAT
TTGGTTCCTT TATGACTTAC TTTTGCTGTA CTGAGGATCA AACCTAGGGT CTCAAGCAGT
CATCACAATT CTCTGTCACT GATCCAGCTC CATTTCTATT TTCTTTTGTC CCGCGCGATC
TCTCGCCAGC- AAGAAAACAC GCTAGGGACA TACGAATCCT TGCTGCAGCC AAAACTTTTA
TTGAATCTTA AGGAGAAGCC CGCGCACCGG ACTGGCGCGG TTTATATACA CCCTAGCACA
GTGCATCCAC A
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Detailed examination of their DNA sequences has confirmed that the six Met-DNA's bear little relationship to one another. C6-DNA shows 86% homology to 102 bp of the rat WAP promoter (Nucleic Acids Res. 12 8685-8697 1984) with a novel duplication of 30 nucleotides of this region. All Met-DNAs contain recognition sequences for transcription factors TCF-1 (EMBO J. 10. 123-132, 1991) and HIP1b (Mol.cell. Biol. 10, 653-661, 1990). Moreover all but one contain recognition sequences for CTCF (Oncogene 5, 1743-1753, 1990), HIPla (Mol.Cell.Biol.10, 653-661, 1990), NF-1L6 (EMBO J. 9 457-465, 1990) and regions of potential Z-DNA (Nature 282, 680-686, 1979),

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with C6-DNA containing a tract of 23 alternating purine-pyrimidine bases. Thus these novel sequences all contain potential regulatory regions for transcription of DNA into mRNA but no known coding or viral-related sequences.

According to an ninth aspect of the present invention there is provided the use of an osteopontin gene as a metastasis inducing transformant.

In one embodiment Met-DNA's, are introduced into a benign rat mammary epithelial cell line Rama 37.

By way of example and to help identify the regulatory function that short stretches of human malignant DNA (precursor to Met-DNA's) may exert on the transfected Rama 37 cells, the mRNA expression of the metastatic transformant rat mammary cell line R37-Ca2-LT1 was compared with its benign parental cell line Rama 37 using subtractive hybridisation techniques. Of the four subtracted clones three corresponded to known rat genes for proteins including osteopontin and one corresponded to a novel rat gene of unknown function. As an example only, transfection of rat osteopontin cDNA into the parental Rama 37 cells produced transformants that induced a high frequency of metastasis compared with vector controls confirming the metastatic capability of

the osteopontin gene as shown in Table 2.

These overall results have established a causal relationship between the Met-DNA's and metastasis on the one hand and the over-or underexpression of certain rat genes, at least one of which is novel, that are linked to the metastatic process in this rat system. Controls with DNA's from nonmalignant, nonmetastatic sources as well as the oncogenes Ha-ras-1, Polyoma Large T Antigen and Polyoma Middle T Antigen failed to induce metastasis establishing the specificity of the inductive processes in this system.

At present the most useful indication of whether a breast or other common cancer will metastasise in the future in a patient is whether the primary tumour has already spread to the local lymph nodes. This test only works on a population basis. For example, in breast cancer, there are many examples of patients with no tumour in the lymph nodes at presentation who later die of metastatic disease and of patients with metastatic deposits in the lymph nodes who live a normal life-span. Thus an accurate test of good predictive value for the occurrence of metastases would be important in selecting those patients for vigorous conventional chemotherapeutic treatments without causing the potentially harmful side-effects in those patients who do not need this treatment.

According to a tenth aspect of the present

invention there is provided a probe specific to a regulatory DNA capable of inducing metastasis.

By specific is meant hybridises to any target DNA under suitable salt and temperature conditions to allow detection of identical or related DNA molecules.

Preferably the probe is provided as part of a kit which may additionally comprise one or more of the following: a colour indicator; an oligonucleotide primer; materials for gel analysis, and/or materials for DNA transfer or hybridisation.

The Met-DNA sequences may be detected in tumour or biopsy specimens by standard Southern blotting, PCR-based or in-situ techniques to identify those patients at risk from metastatic disease. Physical methods of detection based on imaging techniques may also be possible. Expression of metastasis - inducing genes may be detected by standard mRNA hybridisation PCR amplification or by antibodies specific for the gene-product.

According to a eleventh aspect of the present invention there is provided a medicament adapted to target a regulatory DNA capable of inducing metastasis as claimed in any of claims 7 to 13.

In one embodiment such Met-DNA's, metastasis-inducing genes or fragments thereof, could be

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targeted in the cancer cells to excise or block their function using synthetic oligonucleotides based on a knowledge of the sequence of the Met-DNA's, metastasis-inducing genes or fragments thereof, of the invention.

In another embodiment such Met-DNA's, metastasis-inducing genes or fragments thereof, may be targeted for treatment using standard antibody and antisense mRNA/ribozyme techniques for detection and for destruction, respectively.